AN INVESTIGATION INTO THE FERMENTATIVE ACTIVITIES OF THE ACIDURIC BACTERIA *

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The wide use of the bacillus bulgaricus, both topically and by mouth, to control intestinal putrefaction has within the last few years given rise to a number of investigations aiming to give a definite classification to this group of lactic acid bacilli. It would seem that this could be satisfactorily accomplished by a systematic examination of their prominent cultural characteristic, namely, the fermentation of carbohydrates. That this means of separation has not been successfully used heretofore is probably due to the assumption that these bacteria will not grow on the usual laboratory media, though mention is made by the earlier workers of growth in glucose and lactose broth.

According to Grigoroff,[1] the bacillus bulgaricus attacks mannite, saccharose, maltose, and lactose, but not rhamnose, dulcite, or sorbite; according to Cohendy,[2] the bacillus attacks lactose, maltose, saccharose, levulose, and dextrose. In a later work, Bertrand and Duchacek[3] state that this bacillus ferments dextrose, mannite, galactose, levulose, and lactose, but does not attack arabinose, xylose, sorbite, maltose, and saccharose. The lack of agreement exhibited by these findings make them of doubtful value in the identification of this bacillus.

A similar and probably closely related organism is that isolated by Moro from infants' stools and referred to by him as the bacillus acidophilus. The similarity of behavior both biochemically, and as far as was known, culturally, of the acidophilic and bulgaricus groups led several writers, notably Rodella[4] in 1908, and Heinemann and Hefferan[5] in 1909, to conclude that they are identical. Although a survey of the literature and a study of their properties leaves no doubt as to their common membership in the group of non-gas-forming, acid-resisting, lactose-splitting bacteria, there exist, nevertheless, well marked cultural differences that set them apart. These differences form the subject of this paper.

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1. *Rev. med. de la Suisse romande,* 1905, 25, p. 714.
2. *Compt. rend. Soc. de biol.,* 1906, 60, p. 558.
3. *Ann. de l'Inst. Pasteur,* 1909, 23, p. 402.
4. *Centralbl. f. Bacteriol.,* Abt. I, O., 1901, 29, p. 717.
5. *Jour. Infect. Dis.,* 1909, 6, p. 304.

The literature relating to the bulgaricus type of bacillus has been so often summarized that a repetition at this time seems unnecessary and the reader interested in that phase of the subject is referred to the articles of Heinemann and Hefferan,[6] White and Avery,[7] and Kuntze.[8] The state of the literature with respect to the bacillus acidophilus is much the same as in the case of the bacillus bulgaricus; it is recognized as a fermentative organism but apparently little is known in regard to its action on different sugars. According to Moro,[9] the bacillus acidophilus is an acid-resisting bacillus that grows poorly in the usual media and forms irregular colonies on agar. It grows at body temperature and also at 20 C. It is an acid former and coagulates milk. The bacillus acidophilus of Finkelstein has essentially the same cultural characteristics as Moro's bacillus with the exception that it does not coagulate milk, as stated by Lehmann and Neumann.[10] Rodella[11] described a strain exhibiting branching and streptobacillus forms which gave colonies of different shapes on agar plates. Mereshkowsky,[12] who studied the distribution of this bacillus in the animal kingdom, recognizes two types of colonies formed by it, a round, compact, even-edged type, which he designates as Type 1, and one with a filamentous border, Type 2. He found no cultural difference between them. He made use of the following reactions in their identification: they live one to three days in 1 to 0.5 per cent acetic or lactic acid broth; they form the two mentioned types of colonies; they are Gram-positive, and they cause agar to become cloudy.

There seems to be no doubt of the wide distribution in nature of these bacteria and there is likewise no question as to their occurrence among the intestinal flora. Podajezky,[13] who isolated them from bottle-fed and breast-fed infants, from a 10-year-old girl and from a man of 34, showed that the bacillus acidophilus exists in the intestinal tract regardless of age and variety of food. Mereshkowsky isolated it from a long series of animals ranging from mollusks to man.

The general characteristics of both the bulgaricus and acidophilus types of bacilli may be summarized as follows: They are bacilli of varying lengths, occur singly or in chains or threads and develop under both aerobic and anaerobic conditions. Typically they stain Gram-positive but old cultures may be Gram-negative. They produce acids from carbohydrates but do not form gas. They do not form spores. They survive and multiply in media containing considerable amounts of acid. Colony formation, the coagulation of milk, and action on carbohydrates are considered in detail further along.

Since Heyman in 1898 used acetic acid in dextrose broth in isolating a bacillus of the acidophilic type, many authors have followed his example and in this investigation a medium of the same general composition has been used, viz.,

6. *Ibid.*
7. *Centralbl. f. Bacteriol.*, I, O., 1910, 25, p. 161.
8. *Ibid.*, II, 1908, p. 161.
9. *Wien. klin. Wchnschr.*, 1900, 5, p. 114.
10. Bacteriologie, München, 1907.
11. *Centralbl. f. Bacteriol.*, I, O., 1901, 29, p. 717.
12. *Ibid.*, 1905, 39, p. 380.
13. Quoted by Mereshkowsky.

meat-peptone broth containing 2 per cent. glucose and 0.3 per cent. acetic acid. A little of the substance under investigation was seeded into a tube of the broth and incubated for forty-eight hours. One-half c.c. of this culture was then seeded into another tube, and again incubated. After a third seeding and incubation, a loopful of the broth was streaked on the surface of an agar plate. The triple seeding was made for the purpose of eliminating bacteria other than those of the type desired. This is the procedure recommended by Kendall.[14] The agar used for plating was of the usual kind containing 1.5 per cent. of agar and 2 per cent. dextrose, but with no adjustment of its acidity. The growth obtained on this agar was fully equal to that on dextrose whey agar. Later the procedure recommended by Salge[15] was adopted and 0.2 per cent. of sodium oleate added to this medium. As this oleate agar gave a better growth than the plain glucose agar described above, it was used throughout the latter part of the work. Its reaction varied from 0.8 to 1 per cent. The agar plates were incubated for forty-eight hours before the colonies were picked off. In a series of about 100 isolations only four gas formers were found; streptococci were few and aside from these and an occasional growth of yeasts, none but acid-resisting bacteria grew on the plates. The sources of material are given in Tables 1 to 3.

Aside from milk, the only medium used in the differential tests was unneutralized meat-peptone sugar-free broth with the addition of various carbohydrates to the amount of 2 per cent. The initial reaction of the broth varied from +2.2 to +2.8 per cent. Since this variation could not have affected the activity of the bacteria to an appreciable extent, and since the composition of the original broth could not be controlled, nor could a slight degree of hydrolysis be prevented during sterilization and incubation, the various lots of broth were considered in close enough accord for the purpose of this investigation. The broth was sterilized in bulk in the Arnold sterilizer and care was taken to prevent more than a very slight hydrolysis of the carbohydrate. The medium was then tubed in 9 c.c. amounts, and after a preliminary incubation test for sterility the tubes were seeded with a loopful of a forty-eight hour culture of the organisms to be examined, and incubated for five days at 37 C. Five c.c. of each culture were then titrated in the cold against N/10 NaOH, using phenolphthalein as an indicator. It will be observed that this medium is in every sense a "usual" one and it is difficult to see why it has not been used before for the purpose of differentiation. It is true that in the case of organisms of the bulgaricus type visible growth may not appear before forty-eight hours, but it is likewise true that in the presence of suitable sugars there is a measurable degree of fermentation at or before this time. Cultures in this broth have remained viable for a month or longer when kept in the ice chest.

Milk was tubed in 10 c.c. amounts and incubated for a period of 10 days. When clotting occurred the contents of the tube were worked up into a smooth mass and care taken to avoid air bubbles in pipetting the medium for titration. In Tables 1, 2 and 3 Mereshkowsky's terms have been used to indicate the type of colony formed, and the bacilli themselves are classified according to their reaction to Löffler's stain as suggested by White and Avery. In these tables the acid is calculated on the basis of 94 per cent. lactic and 6 per cent. volatile acid. These are the values determined by Heinemann and Hefferan.[16]

14. *Jour. Med. Research,* 1910, 22, p. 153. In this article Kendall introduces the name "aciduric" to take the place of "acidophilic."
15. *Jahrb. f. Kinderh.,* 1904, 59, p. 309.
16. *Jour. Infect. Dis.,* 1909, 6, p. 304.

Table 1 shows that the bacteria of this group all form colonies that correspond to Mereshkowsky's Type 2. Milk was coagulated as a rule before twenty-four hours at 37 C., and there was a large production of lactic acid during the six-day period. The action on maltose was so slight that it may be regarded as negative, and in one case there is a distinct reduction in acidity. With saccharose there was a slight acid production in two instances and a marked one in a third. In the column headed "Type of B. Caucasicum (White and Avery)" the strains are classified according to their reaction to Löffler's methylen blue. According to these authors those that are rapid fer-

TABLE 1.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 1

Culture	Source	Type of Colony (Mereshkowsky)	Type of B. Caucasicum (White and Avery)	Total Acidity Percentage of Normal Acid Six Days in Milk	Percentage of Fixed Acid in Terms of Lactic Acid	Percentage of Normal Acid in Maltose Broth, Five Days	Percentage of Normal Acid in Saccharose Broth, Five Days
Museum	Amer. Museum Nat. His.	2	A	26.3	2.198	0.0	0.1
SK.	Fairchild	2	A	25.3	2.114	0.0	0.0
580	Nat. Hist.	2	A	26.5	2.215	0.0	0.1
582	Nat. Hist.	2	A	25.7	2.147	0.0	2.4
624	Nat. Hist.	2	A	20.9	1.747	0.0	0.0
$Bulgar_1$	Milk (fermented)	2	B	13.0	1.087	0.2	0.2
$Bulgar_2$	Milk (fermented)	2	B	14.8	1.237	0.1	0.1
Zoo	Milk (fermented)	2	B	14.4	1.199	0.0	0.0
B.B.	Drug Store	2	A	25.4	2.122	0.0	0.2
Massolin	Lederle	2	A	11.4	0.953	—0.4	0.4
Bulgara	Tablet	2	A	28.0	2.319	0.2	0.2
$Bacilline_2$	Tablet	2	A	24.6	2.056	0.0	0.6

menters forming inactive lactic acid and staining solidly are called Type A, while those bacilli that are less active, forming levorotary acid and exhibiting purplish granules when stained are termed Type B. In the present work only the staining reaction was made use of in separating these two types. Culture "Massolin" showed a lower acid production than any of the Type B strains, though this is the only exception. It will be noticed that all three of the saccharose fermenters are of Type A.

Microscopic observation showed the organisms of this group to be Gram-positive long bacteria. In older cultures Gram-negative forms occur, and in one instance the buds and stems mentioned by White and Avery were found. Branch and string formations were noted, but there were no short streptobacilli in this group.

The bacteria included in Table 2 form both types of colonies. Milk is coagulated in from forty-two hours to six days. The lactic acid production varied from 0.201 per cent. to 1.304 per cent., the maximum acidity here being about one-half as great as the maximum of Group 1. As the table shows, the bacilli were isolated from various sources. Both long and streptobacilli occurred, and with the exception of culture "S $11/24$" in maltose and saccharose, and culture "Jam" in saccharose, the acid production was marked. Although the limit for clotting was set at six days most of the strains included in the above table caused solidification of the milk before ninety-six hours. In no instance was much whey expressed from the clot.

TABLE 2.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 2

Culture	Source	Type of Colony (Mereshkowsky)	Type of Bacillus	Total Acidity Percentage of Normal Acid Six Days in Milk	Percentage of Fixed Acid in Terms of Lactic Acid	Percentage of Normal Acid in Maltose Broth, Five Days	Percentage of Normal Acid in Saccharose Broth, Five Days
K	Typhoid stool	1	Streptobacillus	12.5	1.045	2.5	3.6
Jam	Saliva	1	Long	9.1	0.753	4.6	0.2
G	Typhoid stool	1	Long	8.5	0.710	8.0	10.5
G_1	Putrefactive stool	1	Long	4.2	0.350	4.3	2.0
R_2a	Normal stool	1	Streptobacillus	12.8	1.070	6.0	5.0
HF_9	Typhoid stool	2	Long	8.0	0.669	4.2	1.2
HF_7	Typhoid stool	1	Long	5.6	0.468	8.2	5.8
HF_3	Typhoid stool	2	Long	4.0	0.334	1.2	1.2
$S11/24$	Typhoid stool	2	Long	8.6	0.719	0.3	0.2
Boh_2	Normal stool	1	Streptobacillus	11.8	0.986	0.8	0.7
Boh	Normal stool	2	Long	3.6	0.301	1.1	2.0
DGC	Typhoid stool	2	Streptobacillus	15.6	1.304	3.5	3.1
L	Typhoid stool	1	Long	5.8	0.485	5.1	4.0
Mt.	Normal stool	1	Long	6.1	0.510	8.4	3.6
Z_2	Fermented milk	2	Long	3.6	0.301	1.9	1.9
Sim	Normal stool	1	Long	10.4	0.869	0.6	1.2
Fly	Intestine of fly	2	Long	2.4	0.201	1.2	1.7
DGC_2	Typhoid stool	1	Long	13.2	1.102	6.0	4.8
B	Typhoid stool	1	Streptobacillus	2.0	0.167	4.4	0.7
R_2	Normal stool	1	Streptobacillus	11.4	0.953	4.9	6.0
HF_4	Typhoid stool	1	Long	4.0	0.334	3.4	1.2

It will be seen on reference to the table that in milk both the most and least active strains are streptobacilli and that these form both types of colonies. Both long and streptobacillus forms showed bipolar staining organisms with Löffler's stain. Although the time of clotting is of some value in differentiating the members of this group from those of Group 1, the essential difference is their active splitting of maltose, and to a less extent that of saccharose.

In this group (Table 3) we have the bacilli that do not clot milk, and the acid production in no instance rises above 2.3 per cent. normal. Both types of bacilli and both types of colonies were present.

The tables show that all of the strains investigated fell naturally into three groups according to their action on milk or maltose broth. The organisms of Table 1 were in some instances pure cultures, in others isolated from lactic acid bacilli tablets or from fermented milks. None of the bacteria isolated from other sources were of this type. The members of Group 2 sometimes showed an acidity in milk nearly

TABLE 3.

FERMENTATIVE ACTIVITY OF BACTERIA OF GROUP 3

Culture	Source	Type of Colony (Mereshkowsky)	Type of Bacillus	Total Acidity Percentage of Normal Acid Six Days in Milk	Percentage of Fixed Acid in Terms of Lactic Acid	Percentage of Normal Acid in Maltose Broth, Five Days	Percentage of Normal Acid in Saccharose Broth, Five Days
F	Typhoid stool	1	Long	0.3	0.025	8.8	10.4
S	Putrefactive stool	1	Long	2.3	0.192	1.8	1.8
Kor	Typhoid stool	1	Long	1.3	0.109	6.0	8.0
C	Typhoid stool	1	Streptobacillus	0.3	0.025	9.4	6.6
Milk	Pasteurized milk	1	Long	0.7	0.059	8.8	8.4
D	Normal stool	1	Long	0.6	0.050	7.3	9.3
P	Typhoid stool	1	Long	1.0	0.084	5.4	0.5
HF	Typhoid stool	1	Long	0.2	0.017	6.5	7.7
CF	Typhoid stool	2	Long	1.8	0.151	1.2	1.7
Tej	Saliva	1	Long	0.1	0.008	9.2	4.1
Wil	Saliva	1	Streptobacillus	0.7	0.059	9.8	8.5
Rat	Rat feces	1	Long	0.4	0.033	10.3	10.3
CF_2	Typhoid stool	1	Long	0.0	0.0	10.4	8.9
HF_8	Typhoid stool	1	Long	0.8	0.067	2.0	0.2
G_2	Putrefactive stool	2	Long	0.0	0.0	7.3	6.6
Wrgt	Normal stool	1	Long	0.8	0.067	5.1	6.7
$Wrgt_2$	Normal stool	1	Long	1.2	0.101	5.2	6.4
Tas	Normal stool	1	Long	1.2	0.101	8.8	0.0
Bacilline	Tablet	2	Long	0.0	0.0	3.4	4.0
R	Normal stool	1	Long	1.0	0.084	1.8	4.1
O. T.	Normal stool	2	Long	2.2	0.184	1.4	1.0

as great as that of some of the organisms of Group 1, but they were sharply set off from it by their action on maltose. In Group 3 milk is not coagulated, and the action on both maltose and saccharose on the whole is greater here than in either of the preceding groups.

It would appear that the bacillus bulgaricus[17] forms but one type of colony while the bacteria of the "acidophilus" variety form two. On what the colony variation in the latter depends the writer does not attempt to say, but neither the opinion of Sandberg,[18] that it is due

17. The writer is indebted to Prof. C. E. A. Winslow, American Museum of Natural History, New York City, for the pure cultures of the bacillus bulgaricus.

18. *Ztschr. f. klin. Med. Berl.*, 1903, 51, p. 80.

to increased acid production, nor that of White and Avery,[19] that it is dependent on the composition and consistency of the medium, seems to explain the variation.

Tested in milk at room temperature, members of these groups showed widely varying degrees of activity. Only one culture in Group 1 clotted the milk in fourteen days. Group 2 showed greater activity, some strains clotting in six or seven days. In Group 3 culture "Milk" clotted in fourteen days though it did not do so at 38 C.

Tables 4, 5 and 6 show the effect of these bacteria on a series of five carbohydrates after five days at 37 C. For convenience the columns showing the action on maltose and saccharose are reproduced in these tables.

TABLE 4.

THE ACTION OF GROUP 1 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Mannite Broth
Museum	5.7	5.9	0.1	0.0	0.5	0.4
SK	16.0	13.9	0.0	0.0	2.4	0.2
580	7.4	7.5	0.1	0.0	2.3	—0.2
582	5.9	3.1	2.4	0.0	0.9	0.2
624	5.4	0.9	0.0	0.0	0.7	—0.2
Bulgara	0.4	3.6	0.2	0.2	0.1	0.0
$Bulgar_2$	1.3	0.8	0.0	0.1	—0.4	0.2
Zoo	4.5	2.4	0.0	0.0	—0.3	0.2
B. B.	2.1	2.0	0.2	0.0	0.0	0.4
Massolin	8.6	5.8	0.4	—0.4	0.2	—0.2
Bulgara	1.8	1.6	0.2	0.2	0.2	0.3
$Bacilline_2$	8.4	3.4	0.6	0.0	0.0	—

It is evident from this table that these strains have a decided preference for dextrose and lactose; in only two or possibly three instances was saccharose attacked. Seven of the twelve strains did not attack levulose, and mannite showed only a very slight fermentation. In those carbohydrates that were but slightly acted on there were occasional instances in which there was a reduction in the acidity. Duplicate tests confirmed this and the same effect occurred elsewhere as can be seen by reference to the tables. Table 5 shows the results with the organisms of Group 2 on the same sugars.

19. *Centralbl. f. Bacteriol.*, 1910, 25, p. 161.

A comparison of this table with Table 2 shows that the acid production in glucose broth in most instances compares very well with that in milk. Lactose, saccharose, and levulose are utilized by all or nearly all of its members, and the differential value of maltose is strongly brought out. Culture "S $11/24$" is the only organism whose place in this group may be questioned. It was placed here because of its rather slow coagulation of milk—seventy-two hours at 38 C. It is interesting to note that all of the mannite fermenters fall within this group, the sole exception being culture "Wil" in Group 3.

TABLE 5.

THE ACTION OF GROUP 2 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Mannite Broth
K	12.2	8.4	3.6	2.5	11.3	4.1
Jam	7.0	6.7	0.2	4.6	10.1	1.7
G	7.7	6.7	10.5	8.0	10.0	1.9
G_1	0.5	1.4	2.0	4.3	8.2	1.8
R_2a	7.1	5.0	6.0	3.4	9.1	3.8
HF_9	3.3	1.2	1.2	4.2	7.1	1.4
HF_7	7.5	5.8	5.8	8.2	8.5	0.0
HF_4	4.4	2.4	0.2	3.4	3.3	2.0
HF_3	1.0	0.2	1.2	1.2	0.7	0.6
$S11/24$	1.4	1.2	0.2	0.3	0.6	1.0
Boh_2	9.0	8.0	0.7	0.8	10.0	2.8
Boh	2.8	1.8	2.0	1.1	1.2	0.0
DGC	10.0	8.4	3.1	3.5	10.4	2.0
L	5.8	4.0	4.0	5.1	5.8	2.6
Mt.	3.9	7.7	3.6	8.4	9.7	0.8
Z_2	0.8	3.2	1.9	1.9	0.6	0.0
Sim	8.2	8.0	1.2	0.6	11.0	1.8
Fly	2.4	1.6	1.7	1.2	1.4	0.0
$D.GC_2$	7.9	4.6	4.8	6.0	9.6	2.6
B	8.6	4.4	0.7	4.4	8.4	1.4
R_2	7.3	4.4	6.0	4.9	9.1	3.8

Table 6 contains the members of Group 3. Although thirteen out of twenty of these strains showed marked acid production in lactose broth the acid formed in milk was very much less.

From the experiments detailed it seems that the bacilli of the bulgaricus type differ in some important essentials from those usually included under the term "acidophilus." To the writer it appears probable that the bacillus bulgaricus is a milk bacillus, and that its appearance in the intestine practically never occurs unless it has been ingested in enormous numbers. Bacteria of the acidophilic type, on

the other hand, are normal inhabitants of the digestive tube. The question of the survival of the bacillus bulgaricus in the intestine in the light of its identification by means of the maltose reaction will be considered elsewhere.

In addition to the media mentioned above, cultivation of these bacteria was attempted in sugar-free broth and agar. With the exception of a very faint growth in the case of culture "Kor" of Group 3 none of the strains grew in sugar-free broth. The acidity of this broth was + 2.8 to phenolphthalein. On sugar-free agar, however,

TABLE 6.

THE ACTION OF GROUP 3 ON CERTAIN CARBOHYDRATES

Culture	Percentage of Normal Acid in Dextrose Broth	Percentage of Normal Acid in Lactose Broth	Percentage of Normal Acid in Saccharose Broth	Percentage of Normal Acid in Maltose Broth	Percentage of Normal Acid in Levulose Broth	Percentage of Normal Acid in Mannite Broth
F	10.4	9.8	10.4	8.8	12.5	0.0
S	3.2	1.5	1.8	1.8	3.1	—1.2
Kor	4.6	7.9	8.0	6.0	8.1	0.0
C	3.4	11.5	6.6	9.4	3.1	0.1
Milk	4.1	0.9	8.4	8.8	7.9	0.1
D	6.6	4.6	9.2	7.3	8.4	—0.4
P	4.0	3.2	0.5	5.4	7.2	0.4
HF	7.8	0.2	7.7	6.5	8.0	0.0
CF	1.0	1.0	1.7	1.2	4.4	0.0
Te	4.4	5.7	4.1	9.2	13.8	0.2
Wil	6.0	6.3	8.5	9.8	15.0	3.2
Rat	10.1	6.6	10.3	10.3	6.1	0.2
CF_2	11.0	11.0	8.9	10.4	2.8	0.0
G_2	7.9	6.8	6.6	7.3	1.1	0.4
Wrgt	5.1	4.0	5.8	5.1	6.7	0.2
$Wrgt_0$	0.9	5.6	2.4	5.2	6.4	0.0
Bacilline	0.6	1.4	4.0	3.4	2.6	0.3
O. T.	0.4	1.0	1.0	1.4	1.0	0.2
Tas	8.5	6.4	—0.2	8.8	1.5	0.4
Ri	1.8	0.6	4.1	1.8	2.0	—0.2

different results were obtained. Although after seventy-two hours the members of Group 1 showed no growth, those of Groups 2 and 3 showed fair development in every instance, though the colonies were small. This agar had a reaction of + 1.

In order to study the production of cloudiness in agar, puncture cultures were made in oleate agar. This clouding was not a constantly occurring characteristic, though it appeared in all three groups. In Group 1, in those instances in which it did happen, the cloudiness appeared after seventy-two hours' incubation in the lower part of the tube and in the region of the puncture, spreading outward and upward

until the end of six days when the agar was uniformly clouded. Among the members of Groups 2 and 3 the cloudiness started earlier and spread evenly. Although a good growth was obtained in every instance, some strains showed not the slightest cloudiness even after six days at 37 C.

In addition the effect of these bacteria on raffinose was studied. This sugar was attacked in a greater or less degree by all of the bacilli of Groups 2 and 3 but those of Group 1 did not act on it.

CONCLUSIONS

From the facts brought out in this investigation the writer is of the opinion that there are three varieties of bacilli in the division of acid-resisting bacteria, at least two of which are of constant occurrence among the fecal organisms:

Variety A, which clots milk but has no action on maltose; Variety B, which clots milk and ferments maltose; and Variety C, which ferments maltose but does not clot milk.

Bacilli of the type of the bacillus bulgaricus may be cultivated in unneutralized meat-peptone broth containing a suitable carbohydrate.

In broth the bacillus bulgaricus does not ferment maltose, and may be differentiated from the other acid-resisting organisms by this characteristic.

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